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Nutrient synchrony: Sound in theory, elusive in practice[1]

M. B. Hall*[2] and G. B. Huntington†

*U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI 53706; and
†Department of Animal Science, North Carolina State University, Raleigh 27695

ABSTRACT: The concept of improving animal performance by going beyond simply meeting requirements and synchronizing ruminal availability of protein and energy has been with us for at least 3 decades. Although theoretically appealing, research and field results have not supported this approach to diet formulation. Why? Essential to successful ruminal synchrony is the ability to predict available amounts and fates of diverse substrates. The substrates come from varied sources; their efficiencies of use and yields of products are affected by inherent properties, interactions, transformations, and passage. However, substrate quality and availability in the rumen are affected only in part by diet. For example, NPN, true protein, and peptides are contributed by diet and intraruminal recycling, with additional endogenous NPN contributions by the cow. Changes in factors that alter the rate or extent of substrate fermentation, such as the rate of passage or ruminal pH, can alter nutrient yield from the rumen and must be accounted for in order for synchrony to work. Our ability to estimate ruminally available substrate is also challenged by normal variation in feed composition and imprecision in component and digestibility analyses. Current in vitro assays may not be adequate to accurately describe the digestibility of feed components in vivo in mixed diets. There are some indications that the amount or pattern of supply of fermentable carbohydrate has a greater impact on microbial production and efficiency than does the pattern of protein supply. Animal responses to modifications in the supply of true protein from the rumen may be masked if additional protein is oxidized by tissues or if AA from endogenous sources cover deficiencies. Animal factors, such as response to immune challenge and sustained damage to tissues, will also affect partitioning of nutrients for production and may alter an animal's response to changes in nutrient supply. With the array of factors internal and external to the diet that must be considered, "synchrony" implies a greater deliberate precision in diet manipulation than may be currently possible to effect. Perhaps we should consider balance. Within the rumen and cow, can we generate conditions so that needed substrates or nutrients are available from the diet or accessible from endogenous resources to meet requirements and enhance productivity and efficiency? This approach involves the whole animal, rather than only the rumen and feed we offer to the cow.

Key words: carbohydrate, fermentation, metabolism, protein, rumen, synchrony

INTRODUCTION

The term "synchrony" is derived from the Greek roots for "together" and "time", to describe simultaneous occurrences. In ruminant nutrition, "nutritional synchrony" most commonly refers to provision of dietary protein (N sources, true protein) and energy (ruminally fermented carbohydrates) to the rumen, such that they are available simultaneously in proportions required or used by ruminal microbes (Figure 1). The concept has a shorter term temporal aspect that sets it apart from simply meeting daily nutrient requirements and preventing gross deficiencies. Theoretically, synchronous nutrient availability should allow more efficient use of nutrients, thus enhancing production of microbial products, increasing nutrient supply to the animal, and potentially improving animal production performance. However, in the 30 yr or more that nutritional synchrony has been explored, it has frequently failed to show improved microbial or animal performance. In a number of cases, asynchronous diets gave animal results that were as good as, or superior to, synchronous diets in ruminal and animal performance measured in growing beef cattle and lactating dairy cattle fed in confinement (Kim et al., 1999b; Valkeners et al., 2004).
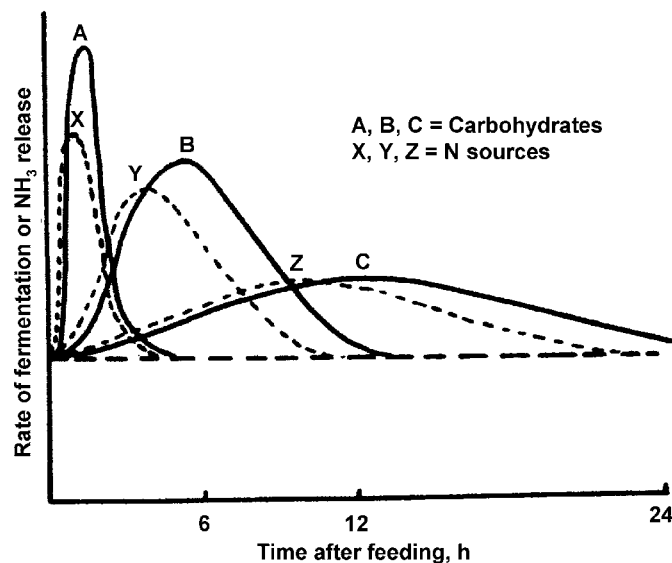
Figure 1. Illustration of the theoretical rumen fermentation rates over time after ingestion of (A) rapidly, (B) moderately, and (C) slowly fermented carbohydrates, and the proposed complementary rumen-ammonia curves (X, Y, and Z, respectively), as required to support microbial protein synthesis. From Johnson (1976) and published with permission of the *Journal of Animal Science*.

Are these results a function of failure of concept, or failure of application?

Successful implementation of nutritional synchrony requires the ability to quantitatively predict the outcome of the ruminal interplay of dietary protein and energy to produce microbial products, as well as the factors that affect partitioning of nutrients by the animal. In both of these areas, consideration must be given to the effects of factors external to the diet. In this paper, we briefly explore a variety of factors that affect our ability to successfully impose nutritional synchrony in ruminant nutrition.

NUTRIENT SUPPLY

The concept of nutritional synchrony presumes that the diet is the major determinant of the quantity and quality of nutrients supplied to the rumen microbial population and to the animal. In reality, multiple ruminal and endogenous pools determine nutrient availability to the rumen and animal. Protein provides a good example (Figure 2). Microbial protein can be produced directly from ruminally degraded dietary protein. In addition, ammonia, AA, and peptides are available from intraruminal lysis and recycling of microbes and from urea that is produced from ruminal and extraruminal contributions of ammonia to ureagenesis. Each of the sources that is not directly derived from dietary components can change the temporal pattern of protein availability from that predicted for the diet alone. And those sources may not be consistent in their supply; a study

in which pectin was infused abomasally in dairy cattle provides evidence that increasing fermentation of carbohydrate in the hindgut has the potential to decrease ruminal ammonia concentrations (Gressley and Armentano, 2005). The hindgut fermentation likely acts as an alternate sink for urea.

Ruminal escape of dietary protein and of microbial protein produced in the rumen each represent a portion of the AA supply available to the animal (Figure 2). Amino acids synthesized by the body and those liberated by tissue mobilization likely allow the animal to fill needs unmet by the dietary supply, at least in the short term, perhaps as long as the resources of the animal allow it or until the nutrient demands are reduced. The availability of nondietary sources of AA could reduce detection of short-term or relatively small inadequacies of dietary AA supply.

In this light, attempts in vivo to show differences in the effect on animal performance of synchronous and asynchronous provision of nutrients to the rumen may have been fighting an uphill battle against homeostasis, "the tendency of the body to seek and maintain a condition of balance or equilibrium within its internal environment, even when faced with external changes" (Hirsch, 2003). In the interest of survival, it is not surprising that animals can accommodate at least some shortfalls in nutrient supply. For efforts in nutritional synchrony to succeed, the approach taken would have to incorporate consideration of the dietary and nondietary sources of nutrients being synchronized. Focus on supplementation of specific nutrients rather than broad categories of protein and energy may be needed.

A number of studies of ruminal fermentation in vivo and in vitro indicate that the current approach to nutritional synchrony will not provide the desired results. A common theme among these studies is that energy, or more accurately, available carbohydrate, has a significant impact, whereas synchronization does not have a consistent effect. In an infusion study with nonlactating dairy cows fed in confinement, microbial protein supply, measured as excreted purine derivatives, were equally increased ($P < 0.05$) over the basal diet by continuous synchronous (two 6-h infusions begun at feeding time) and asynchronous (two 6-h infusions begun 6 h postfeeding) infusions of sucrose (Kim et al., 1999a). In a similar study with lactating dairy cows offered grass silage and concentrate, continuous and synchronous infusions of maltodextrins gave similar increases ($P < 0.05$) in microbial protein supply over the basal diet (Kim et al., 1999b). A continuous culture study with mixed ruminal microbes that evaluated the direct effects and interactions of 2 concentrations of nonfiber carbohydrates and 2 concentrations of ruminally degradable protein concluded that "the preponderance of significant main effects" demonstrated that fermentations were more likely to be limited by availability of carbohydrate or N than they were to be improved by protein and carbohydrate synchronization (Mansfield et al., 1994). In vitro fermentations with mixed ruminal
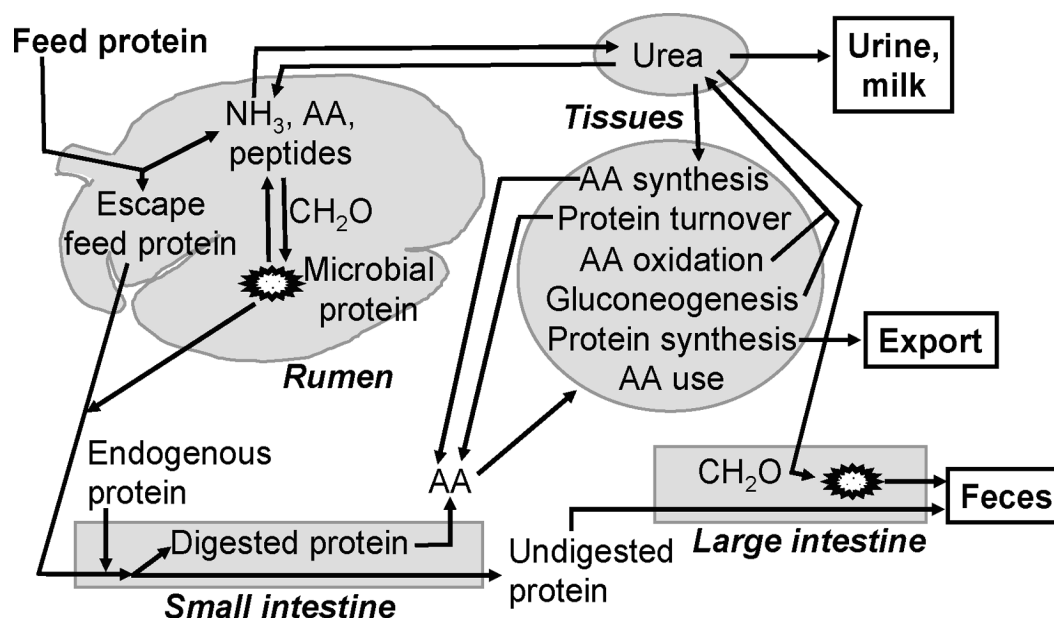
Figure 2. Interplay of dietary, ruminal, and extraruminal sources of N and AA. CH$_2$O = carbohydrates; Export = milk, conceptus, scurf, secretions. Gut or tissue compartments are labeled in bold, italic print.

microbes by Henning et al. (1991) showed that the most rapid rate of energy availability gave the highest bacterial cell production efficiency. They concluded that "synchronization between energy and N availability may be of less importance to bacterial growth efficiency than the energy supply pattern" (Henning et al., 1991). Because there is no endogenous system to ensure a supply of energy to the rumen as there is for N, perhaps we need also consider that the animal may be adapted to ensure availability of one nutrient (protein) to capitalize on another nutrient (carbohydrate) when it is available.

RUMINAL CONSIDERATIONS

In focusing on the ruminal aspects, nutritional synchrony requires description of the supply of fermentable OM and factors that determine its disposition as microbial products (i.e., microbial cells, organic acids, gases, etc.). Accurate measures of DMI are needed to determine total mass of substrate and estimates of rates of passage from the rumen. Information on passage rate is needed to estimate the proportions of substrates that will be fermented ruminally.

Accurate feed sampling and characterization of feed composition are essential to describing diet fermentation and utilization by the microbes. Compositional measurements may include protein and carbohydrate fractions and their rates of fermentation, physically effective fiber, and microbially required nutrients (e.g., sulfur). Estimates of energy alone may not be adequate because carbohydrates differ in their fermentation characteristics. These differences include dissimilar VFA profiles (Strobel and Russell, 1986), and the effects

of pH (Strobel and Russell, 1986) and degradability of N sources (Aldrich et al., 1993) that could affect microbial product output and the adequacy of these products to fill animal requirements. The extent to which feed composition varies over time will affect how precisely treatments can be imposed. This may be of special concern in grazing or commercial feeding situations in which changes in the growing plants or in supplies of forage or commodity feeds have the potential to create ongoing changes in the diets.

Error, or variation, is associated with each analytical measure or prediction that we make. The error associated with each of the measurements required to model nutritional synchrony places limits on the precision with which dietary manipulations can be applied. Availability and selection of analyses to make the needed characterizations may also be a challenge. Because our knowledge of animal biology and ruminal ecology is incomplete, we are also uncertain of the aspects of and most appropriate methods for feed characterization that best describe the dynamic system of the animal. We do not currently have all the methods that we need.

Determination of relative (rapid vs. medium or slow), if not quantitative, rates of ruminal degradation on protein- and energy-supplying substrates is needed to establish the desired dietary amounts of synchronous substrates. These rates, even when determined under carefully controlled conditions, may be qualitative or incorrect when applied to the animal. Effective rates of ruminal carbohydrate fermentation can be altered by changes in pH (which alters the extent of in vitro fermentation of carbohydrates by a fixed time point; Strobel and Russell, 1986), quantity of starch fed (which alters the in vivo rate of starch fermentation; Oba and

Allen, 2003), type of nonfiber carbohydrate supplied (which alters in situ NDF fermentation; Heldt et al., 1999), or storage rather than immediate fermentation of a carbohydrate (sugars and fructans stored as microbial α-glucan; Thomas, 1960). Factors that appreciably modify carbohydrate fermentation must be taken into account to establish protein needs in a synchronous ration. However, our ability to predict the occurrence and quantify the effects of a number of these factors is limited, so our ability to consider them objectively in diet formulation is also limited.

Interactions among ruminally degradable protein and carbohydrate fractions that alter the production or yield of nutrients also have the potential to alter the outcome of attempts at synchrony. Increasing the amount of degradable protein relative to fermented carbohydrate has been shown to increase the yield of microbial protein in vitro (Argyle and Baldwin, 1989), organic acids in vitro (Hall and Weimer, 2007), and organic acids in vivo in dairy cattle (Aldrich et al., 1993) per unit of carbohydrate fermented. The change in yield may be explained by conversion of substrate carbon to products other than organic acids, microbial cells, and gases when microbes experience a relative deficiency of ruminally degradable protein to carbohydrate (Russell, 1998).

The type of dietary carbohydrate provided has the potential to affect microbial protein production indirectly. Increasing the proportion of sugar in diets has been related to decreases in ruminal concentrations of branched-chain fatty acids, which are needed for de novo synthesis of AA by ruminal microbes (Sannes et al., 2002). There is the possibility that reduction in the branched-chain fatty acids could decrease protein production and microbial growth.

PREDICTING POSTRUMINAL NUTRIENT SUPPLY AND USE

If successfully executed nutritional synchrony did alter the supply of nutrients, would it increase animal use of the nutrients for productive purposes? A common presumption is that changes in ruminal degradability, microbial protein yield, or both from the rumen will change the supply of absorbed N; specifically, that increased supply of protein to the abomasum and small intestine will increase AA absorption, and increased AA absorption will improve the production response to the extent that it had been limited by AA supply. However, 2 things may intervene to thwart the presumption. The first is the buffering effect of absorption and transfer of AA. In intensive experiments, postruminal infusion of casein with multicatheterized ruminants increased absorption of AA into blood draining the gut (Huntington and Archibeque, 2000). However, measured absorption rarely meets expectations calculated from intestinal disappearance because of the substantial use of AA by the gut tissues craniad to the small intestine (MacRae et al., 1997).

The second factor that may conceal a putative response to ruminal synchrony is the relatively high contributions of NPN to the overall N flux in ruminants. Transfer of urea N from blood to the lumen of the gut, not including salivary transfer, ranged from 12 to 41% of N intake in cattle across a wide range of diet types and protein intakes (Huntington and Archibeque, 2000). Consumption of semipurified diets that contained from 31 to 84% in vitro undegradable protein did not cause detectable changes in the rates or proportions of dietary N absorbed as ammonia N or AA N in beef heifers (Huntington, 1987). Across the diets, the ratio of the net absorption of AA N:ammonia N by the heifers was 1.6:1. In lactating dairy cows consuming diets containing 15 to 18% CP, the amount of N absorbed as ammonia N was approximately equal to N absorbed in the form of AA; the flux of NPN across the gut (absorption of ammonia plus recycling of urea N) was approximately equal to the amount of N excreted as milk N (Bach et al., 2000; Berthiaume et al., 2006). Therefore, changes in microbial protein production caused by synchrony of ruminal energy and N supply may be masked by the relatively greater fluxes of other nitrogenous compounds.

ANIMAL INFLUENCES: IMMUNE FUNCTION, REQUIREMENTS, DAMAGE, CIRCADIAN RHYTHMS

Our ability to detect the impact of nutrient synchrony or any dietary manipulations on production is affected by animal factors that alter nutrient use. Specifically, immune status, damage to tissue function, and the animal's own metabolic fluctuations may alter response to the diet. The primacy of the immune system in the overall scheme of survival means that even nutrient synchrony that is successful and imposed to improve the animal response can be disrupted by response to an immune challenge. The immune response may be caused by disease, stress, or trauma; the response dramatically increases metabolic demand for AA, usually in the face of depressed feed intake (Sandberg et al., 2006). While tissue catabolism supplies the immediate demand of the immune response, the dietary requirement for the concentrations of energy and protein increase. Acute and chronic immune challenges in rats, pigs, and humans decreased N balance, increased rates of protein turnover, and had specific effects on circulating levels of AA (Reeds and Jahoor, 2001; Kurpad, 2006). Chronic parasitism in sheep causes changes in partitioning of AA among visceral, hepatic, and peripheral tissues (Bermingham et al., 2007), moving AA away from peripheral tissues in favor of parasitized visceral tissues. In what may be a far longer term effect of immune function, neonatal calves that do not receive adequate colostrum at birth had persistently greater prevalence of disease and death until slaughter (Wittum and Perino, 1995). Regression analyses showed deleterious effects of inadequate colostrums on subse-

quent weight gain and feed efficiency, indicating that the "normal" diet of the calves was inadequate to attain the potential growth.

It is open to question whether the long-term response to an immune challenge is related to the immune response, per se, or to damage sustained by the animal. Six months after ruminal acidosis was induced in lambs by ruminally infusing glucose (18 g·kg^{-1} of BW), the fractional rate of ruminal acetate absorption was 13% lower than for the control animals. This indicates that short-term severe insult with acute ruminal acidosis may reduce organic acid absorption for an extended period of time (Krehbiel et al., 1995). Anecdotally, field nutritionists report that in herds of dairy cattle that have experienced ruminal acidosis, it may take 4 to 6 wk after an appropriate diet has been established for herd production to return to levels predicted to be supported by the diet. The alterations in supply and demand related to immune function or functional damage are independent of any imposed dietary manipulations. In research and commercial herds, it is certain that some proportion of the animals experience some degree of immune challenge or damage, and their responses will affect the outcome of dietary manipulations.

Animals are not steady-state systems with small tolerances. Temporal fluctuations internal to the animal may alter the day-to-day responses to manipulations of diet and nutrient supply. For example, circadian rhythms in body temperature and insulin recorded in animals housed in an environmental chamber and maintained on a regular feeding and milking schedule have their own periods and fluctuations that do not appear to be associated with imposed events (Lefcourt, et al., 1999; Figure 3). Under these controlled conditions, animals showed episodic peaks that exceeded the amplitude of the circadian rhythms. Such variation within the animals indicates a system of metabolic controls that need to be considered for external manipulations to succeed.

CONCLUSIONS

As it has been attempted, imposition of nutritional synchrony of protein and energy in the rumen has not consistently generated the expected increases in microbial efficiency and animal productivity. The concept may require a greater deliberate precision in dietary manipulation than is currently possible to effect. It is certain that any hope for success with this concept must involve taking into account the larger picture and complexity of the interaction of the animal, its environment, the ruminal microbes, and the diet, and perhaps making more specific designations of nutrients to be synchronized beyond protein and energy. To this end, we need to verify that relevant biology and interactions have been correctly described, and that we can quantitatively and accurately characterize the factors we wish to manipulate. The array of factors internal and external to the diet that can alter the outcome of dietary
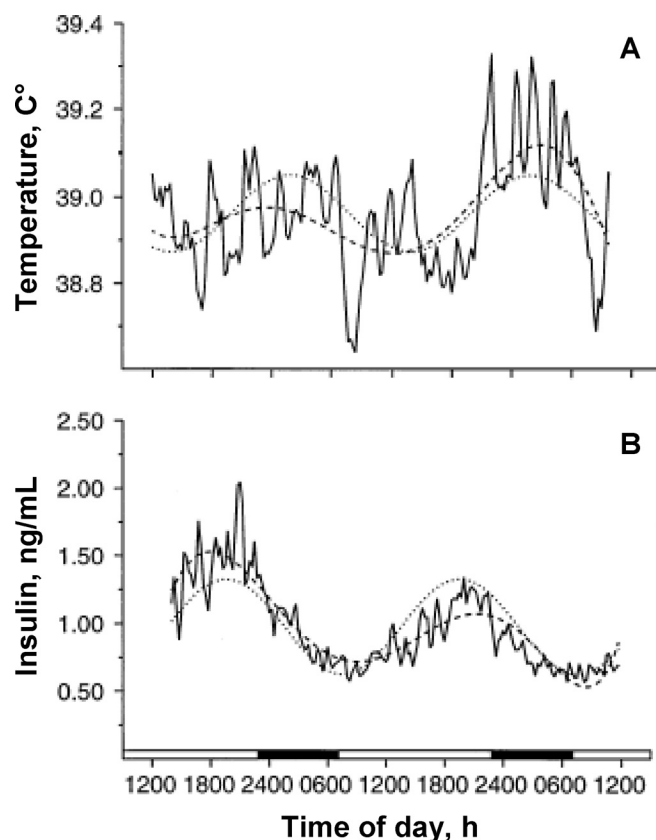
Figure 3. Circadian patterns of (A) body temperature (average of 5 cows) and (B) plasma insulin concentration (average of 6 cows) of lactating dairy cattle recorded over a 48-h period. Insulin was determined on samples taken every 15 min. Animals were housed in environmental chambers maintained at 19°C with lights on from 0700 to 2300, fed daily at 0900, and milked at 0800 and 2000. Graphs show raw data (solid lines), polynomial functions (dashed lines), and cosine functions (dotted lines). From Lefcourt et al. (1999) and published with permission of Elsevier Limited.

manipulations needs to be delineated, and the magnitude of their impact as direct effects and interactions should be accounted for. In the face of animal adaptability, we must honestly ask whether nutritional synchrony in its present form is a sound concept, or if we need to devote our efforts to other dietary and management interventions to enhance animal performance. Perhaps we should consider balance, focusing on generating conditions in the rumen and animal so that needed substrates or nutrients are available from the diet or accessible from endogenous resources to meet nutrient requirements and to enhance productivity and efficiency. This approach involves the whole animal, rather than only the rumen and feed we offer to the animal.

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